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Organellar Channels and Transporters

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Abstract

Decades of intensive research has led to the discovery of most plasma membrane ion channels and transporters and the characterization of their physiological functions. In contrast, although over 80% of transport processes occur inside the cells, the ion flux mechanisms across intracellular membranes (the endoplasmic reticulum, Golgi apparatus, endosomes, lysosomes, mitochondria, chloroplasts, and vacuoles) are difficult to investigate and remain poorly understood. Recent technical advances in super-resolution microscopy, organellar electrophysiology, organelle-targeted fluorescence imaging, and organelle proteomics have pushed a large step forward in the research of intracellular ion transport. Many new organellar channels are molecularly identified and electrophysiologically characterized. Additionally, molecular identification of many of these ion channels/transporters has made it possible to study their physiological functions by genetic and pharmacological means. For example, organellar channels have been shown to regulate important cellular processes such as programmed cell death and photosynthesis, and are involved in many different pathologies. This Special Issue (SI) on Organellar Channels and Transporters aims to provide a forum to discuss the recent advances and to define the standard and open questions in this exciting and rapidly-developing field. Along this line, a new Gordon Research Conference dedicated to the multidisciplinary study of intracellular membrane transport proteins will be launched this coming summer.

Introduction

Ion channels are classically understood to mediate the flux of ions across the plasma membrane in response to cellular stimulation. However, they also reside on intracellular membranes to regulate various organellar and cellular functions as well [1, 2]. Intracellular organelles can be arbitrarily divided into two groups [3]. The first endocytic, secretory, and autophagic group (group I) includes the endoplasmic reticulum (ER), the Golgi apparatus, endosomes, autophagosomes, phagosomes, lysosomes, secretory vesicles, and vacuoles (see **Fig. 1**). Group I organelles mediate cargo transport and exchange materials with each other [4]. There are also cell-type-specific compartments derived from group I organelles, which include synaptic vesicles in neurons [5] and various lysosome-related organelles, such as melanosomes in melanocytes [6]. Group II intracellular organelles include mitochondria, nucleus, chloroplasts, and peroxisomes, which are dedicated to specific cellular functions such as bioenergetics (mitochondria and chloroplasts). Ion channels and transporters are functionally present on the membranes of the aforementioned organelles [1, 2].

A major function of organellar ion transport is to regulate intracellular Ca^{2+} signaling, which plays important roles in both signal transduction and membrane trafficking [1, 2, 4]. Indeed, many group I and II intracellular organelles serve as intracellular Ca^{2+} stores with the luminal Ca^{2+} concentration ($[Ca^{2+}]_{lumen}$) ranging from micromolar (μM) to millimolar (mM), 10 to 5,000-fold higher than the level of resting cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$, ~ 100 nM) [3]. Consistently, many Ca^{2+} channels and transporters are enriched in intracellular organelles [2]. For example, the inositol 1,4,5-trisphosphate receptors (IP3-Rs) are Ca^{2+}-permeant channels in the ER, the primary Ca^{2+} store in the cell [7]. IP3-Rs are the essential signal transduction player in the phospholipase C (PLC) pathway that is stimulated by numerous neurotransmitters and hormones (Mak and Foskett; Ref. [8] in this SI). Additionally, intracellular transport of other ions such as Na^+ and K^+ regulates organellar membrane potential and luminal ionic homeostasis, which are known to affect Ca^{2+} signaling indirectly [2]. For instance, ER K^+ channels are reported to affect both Ca^{2+} uptake and release (Kuum et al; Ref. [9] in this SI).

The last wave of organellar channel research culminated in the identification of two ER-localized Ca^{2+} release channels: IP3-Rs and Ryanodine receptors (RyRs) [7, 10]. Recently, the field has experienced another dramatic development, surmounting technical

limits through new methods like patch clamping of endosomes and lysosomes [11-13], and through molecular identification of channels affecting cell and bioenergetic activities (e.g., the long-sought identity of the mitochondrial calcium uniporter, MCU) [14, 15]. Many new channels and transporters have been discovered in both group I and II intracellular organelles, such as mitochondria, lysosomes, Golgi apparatus, ER, melanosomes, and plant vacuoles. Much of this work is the basis for the reviews in this SI. Included in this series are papers on mitochondrial MCU Ca^{2+} channels (Murgia and Rizzuto; Ref. [16] in this SI), mitochondrial K^+ channels (Leanza et al, Ref. [17] in this SI), mitochondrial permeability-transition-pore (PTP) proteins (Rasola and Bernardi, Ref. [18] in this SI), endosomal Cl channels/transporters (Pusch and Zifarelli, Ref. [19] in this SI), lysosomal NAADP receptors (Galione, Ref. [20] in this SI), lysosomal TRP-type Ca^{2+} channels (Venkatachalam and Zhu, Ref. [21] in this SI). Ion channels in the ER are also discussed, including the IP3R-type Ca^{2+} channels (Mak and Foskett; Ref. [8] in this SI) and various K^+ channels (Kuum et al, Ref. [9] in this SI). Examples of discovery can be drawn from chloroplasts, such as the identification of new membrane transport proteins through proteomics and transcriptomics (Finazzi et al, Ref. [22] in this SI), and the discernment of how subcellular targeting and biogenesis of organellar channels are regulated (Oh and Hwang, Ref. [23]; von Charpuis et al, Ref. [24] in this SI). So far, organellar mechano-sensitive channels are only characterized in yeast cells (Nakayama and Iida, Ref. [25] in this SI). Finally, sperm ion channels are covered to exemplify how electrophysiological studies in non-intracellular organelles can be instrumental (Miller et al, Ref. [26] in this SI).

Common themes emerge upon a collective reading of these reviews. First, improved electrophysiological methods and fluorescence-based functional assays have led to functional identification of new organellar channels. Recent examples include lysosomal TRPML1 (SI Ref. [21]), lysosomal two-pore TPC channels (SI Ref. [20]), and mitochondrial MCU channels (SI Ref. [16]). Second, improved biochemical and system-based methods have led to the discovery of new intracellular channels/transporters, such as mitochondrial MCU channels ([14, 15] and SI Ref. [16]) and metal transporters in chloroplasts (SI Ref. [22]). Third, advances in the understanding of organellar channels/transporters have led to the identification of novel targets for therapeutics.

Examples of new "druggable targets" in this series are lysosomal channels for lysosome storage diseases (LSDs) and mitochondrial channels for cancer (SI Refs. [18, 21]). Together, these studies provided an updated "toolkit" for tackling the difficult study of intracellular channels and transporters. Due to space limitation, many of the recently discovered organellar channels and transporters are not covered in this SI. We first outline common challenges, then discuss the progress in each subfield/organelle, with the focus on the mitochondria, chloroplasts, lysosomes, and plant vacuoles.

Common challenges in studying organellar channels

There are common challenges in studying channels from different intracellular organelles. Unlike plasma membrane channels, whose working environment has been unambiguously defined, the basic information for most organelles has yet to be established, including luminal ionic composition, organellar membrane potential, and lipid composition of the organellar membranes.

Luminal ionic composition varies greatly in different subcellular contexts, adding a layer of difficulty in the task of properly characterizing the function of organellar ion channels. The most relevant luminal ions are Ca^{2+} and K^+. While $[Ca^{2+}]_{lumen}$ is high for the ER and lysosomes, and low for mitochondria, $[K^+]_{lumen}$ is high in the ER, nucleus, and Golgi, but relatively low in mitochondria and lysosomes [2, 27]. Importantly, in small-sized organelles like endosomes and lysosomes, the luminal concentration of one ion must be viewed in the context of other ions and ion-dependent channels/transporters. Due to the enrichment of various ion co-transporters and exchangers in organelles [28], an increase in the permeability of one ion may alter the concentration gradients of others. Hence, unlike their plasma membranes counterparts, organellar ion transporters may have a direct and acute influence on the functions of organellar channels.

What is the membrane potential ($\Delta\psi$, defined as V_{lumen}- $V_{cytosol}$ for comparison) for each organelle? Resting $\Delta\psi$ is around 0 mV for the ER and nucleus, very negative (-150 to -180 mV) for mitochondria, and slightly positive (+ 20 to 30 mV) for the Golgi apparatus, phagosomes, and lysosomes [1, 2, 28]. For plant vacuoles, a membrane potential around +30 mV is assumed, however, it remains unknown whether it can fluctuate in response to changes in environmental conditions [29]. For the chloroplast envelope membrane, a value

of approximately -110 mV has been reported [30]. The ionic permeabilities that set $\Delta\psi$ at rest or upon stimulation remain to be determined.

What are the identified channels and transporters in the organelles? Many ion channels and transporters are reportedly present in the organelles based on molecular expression analysis, pharmacological manipulation, or functional characterization. However, only few of them are supported by strong data in all three aspects. In addition, while some channels are targeted specifically and exclusively to one organelle, others are present in multiple cellular compartments. Hence, for channels present in both plasma membrane and organelles, it is necessary to set up the criteria to define organellar *versus* plasma membrane channels. On the other hand, the fact that phamarcological properties of such channels, located either intracellularly or at the plasma membrane, appear to be the same in many cases renders assigning a definite role to intracellular channels in a given process a difficult task.

The endocytic compartments

Regulation of lysosomal function and trafficking by lysosomal ion fluxes

Lysosomes, acidic vesicles that are filled with Ca^{2+} and hydrolases, mediate the degradation of both endocytic and autophagic cargos [31]. Subsequently, the digested metabolites are transported out of the lysosome via specific exporters or through vesicular membrane trafficking [32, 33]. Lysosomal channels and transporters mediate ion fluxes across perimeter membranes in order to regulate lysosomal ion homeostasis, membrane potential, catabolite export, and membrane trafficking [28]. Deregulation of lysosomal channels may underly the pathogenesis of many Lysosome Storage Diseases (LSDs) and possibly some metabolic diseases [34].

There exist large concentration gradients for Ca^{2+} (~ 5,000 fold), H^+ (~ 1, 000 fold), Na^+ (~ 10 fold), and K^+ (~ 10 fold) [35, 36]. The proton gradient (pH_{lumen}~4.6) is established and maintained by V-ATPase [31]. Cl^- influx regulates lysosomal acidification by providing counter ions for H^+ pumping [37, 38]. $[Ca^{2+}]_{lumen}$ is ~ 0.5 mM for lysosomes [28] , higher than the low micromolar ranges for early and late endosomes [39]. Ca^{2+} efflux from lysosomes is important for signal transduction [28]. Lysosomal Ca^{2+} is also known to regulate multiple steps in lysosomal trafficking, including fusion of lysosomes with

autophagosomes, late endosomes [32, 40], lysosomal exocytosis [36, 41], retrograde trafficking to the Golgi apparatus, and lysosome reformation from the autolysosomes [42] or endolysosome hybrids [32]. The high $[Na^+]_{lumen}$ and low $[K^+]_{lumen}$ may help set the $\Delta\psi$, which, like H^+ flux, may indirectly affect lysosomal Ca^{2+} release [28, 43] . With the exception of V-ATPase, most lysosomal ion transporters have yet to be identified. The Ca^{2+} gradient is thought to be established by a putative Ca^{2+}-H^+ exchanger in the mammalian cells [28]. The molecular identity of this high affinity Ca^{2+} transporter is still unknown. In contrast, in the yeast and plant vacuoles, both Ca^{2+}-H^+ exchanger and Ca^{2+}-ATPase are required for the maintenance of the vacuolar Ca^{2+} store [44].

Although the importance of lysosomal ionic flux has been long appreciated, the ion channels responsible for lysosomal Na^+, K^+, Ca^{2+}, Cl^-, and H^+ fluxes are only beginning to be discovered.

Endolysosomal patch-clamping

The traditional way to study endosomal and lysosomal channels is to reconstitute them into a planar lipid bilayer [45]. However, bilayer studies require a high degree of purity in membrane and protein preparation, and typically do not yield large macroscopic currents. To characterize endosomal and lysosomal channels in their native membranes, the biggest hurdle is their relatively small size (< 0.5 μm in diameter), suboptimal for patch-clamping studies [11, 12]. Recently, this barrier has been overcome by advances in cell biology. Large early endosomes (> 3 μm in diameter) can be formed by expressing mutant forms of trafficking proteins [11]. Alternatively, late endosomes and lysosomes, can be selectively enlarged using small molecule vacuole-enlargement reagents, such as vacuolin-1 [12, 13, 46]. Four different configurations can be made for endolysosomal electrophysiology: endolysosome-attached, whole-endolysosome, luminal-side-out, and cytoplasmic-side-out [11, 12]. Genetically-encoded ion indicators that are targeted to endolysosomes may be employed to study the flux of ions. However, the whole-endolysosome technique represents the most powerful method to study ion channels in the endosomes, lysosomes, and other related intracellular vesicles, including phagosomes and melanosomes [13, 46, 47].

Lysosomal conductances

As H^+ and Ca^{2+} are 1,000-5,000 times more abundant in the lysosome lumen than in the cytosol, lysosomal Ca^{2+} and H^+-permeant channels must be tightly regulated. Meanwhile, the high Na^+ and K^+ gradients across lysosomal membranes suggest the existence of selective Na^+ and K^+ channels in the lysosome. Using a lysosome patch-clamp technique [12], multiple lysosomal conductances have been functionally characterized, including I_{Na}, I_{Ca}, I_{Fe} and I_{Cl} [12, 13, 46, 48-50]. I_K and I_H have not been fully characterized. In addition, endogenous I_{NAADP} has been reported in mouse fibroblasts [51]. Among these conductances, TPCs have been molecularly identified to encode I_{Na} [27] and TRPMLs to encode I_{Ca} [12] (see **Fig. 1**). CLC-7 is presumed to encode I_{Cl} [38] (see **Fig. 1**).

Lysosomal Ca^{2+} channels

Mucolipin TRPs (TRPML1-3) are the principle Ca^{2+} release channels in lysosomes. TRPML1 is a key regulator of most lysosomal trafficking processes [40, 52], and human mutations of *TRPML1* cause lysosomal storage and Type IV Mucolipidosis (ML-IV) [53, 54] (Also see SI Ref. [21]). Using whole-endolysosome patch-clamp technique, TRPML1 is demonstrated to be a late endosome and lysosome-localized, Ca^{2+} and Fe^{2+}/Zn^{2+} dually-permeable channel activated by an endolysosome-localized phosphoinositide, i.e. $PI(3,5)P_2$ (SI Ref. [21]). The regulation of TRPML1 by $PI(3,5)P_2$ provides an example of compartment-specific regulation of organellar channels.

The cell biological roles of TRPML1 were uncovered with the aid of membrane-permeable synthetic agonists [55, 56]. Using Mucolipin Synthetic Agonist 1 (ML-SA1), which robustly activates TRPML1 at low micromolar concentrations [56], TRPML1 is found to be a primary regulator of lysosomal exocytosis [47]. TRPML1-mediated lysosomal exocytosis is required for the phagocytic uptake of large particles in macrophages [47] and repair of plasma membrane damage in skeletal muscle [57]. Loss-of-function mutations in *TRPML1* cause ML-IV, a LSD manifested by mental retardation, muscular dystrophy, and constitutive achlorhydria [54, 57]. In addition, TRPML1's role may also be extended to other LSDs [56], in which TRPML1-mediated lysosomal Ca^{2+} release and lysosomal trafficking are partially blocked [56].

Two-pore channels (TPC s) are also thought to be lysosomal Ca^{2+} channels (SI Ref. [20]). TPCs are localized in the lysosomes, and overexpression of TPCs increases NAADP-activated Ca^{2+}-release [58]. In whole-endolysosome recordings, I_{NAADP} was increased in TPC-overexpressing cells, but abolished in TPC2 KO cells [51, 59]. TPC KO mice exhibit susceptibility to liver disease and impaired starvation endurance (SI Ref. [20]).

P2X4 channels are recently identified to be ATP-activated Ca^{2+}-permeable channels in the lysosomes of Cos1 cells [60]. P2X4 proteins are localized in the lysosome, and overexpression of P2X4 results in large non-selective cationic currents activated by luminal ATP and alkalization [60]. The physiological significance of lysosomal P2X4 channels remains to be established.

Lysosomal Na^+ channels

Whole-endolysosome TPC currents are highly selective for Na^+ over K^+ or Ca^{2+} [27, 50]. TPC channels are regulated by $PI(3,5)P_2$ [46], membrane voltage [50], and cytoplasmic Mg^{2+}/ATP [49, 59]. Given TPC's high permeability to Na^+, regulation of TPC currents may provide mechanisms to rapidly change lysosomal $\Delta\psi$. Under conditions when $PI(3,5)P_2$ levels are high but ATP levels are low, endolysosomes that lack TPCs have a less depolarized (luminal-less-positive) $\Delta\psi$ [49].

Endosomal and phagosomes

Several CLC (CLC3-7) proteins are localized in the early and late endosomes [38]. Although the biological functions of CLCs in endosomes have been clearly established, their channel or transporter properties are characterized mostly at the plasma membrane (SI Ref. [19]). Therefore, endosomal patch-clamping is needed to characterize CLCs in their native settings. Several other proteins are also present in the early endosomes, including TRPML3, TPC1, and also possibly TRPV2 [11, 52, 61]. However, their roles in early endosomal functions are unclear.

Whole-phagosome patch-clamping techniques have been recently developed in macrophages [47]. This technique should be employed to study phagosomal conductances, including those are already known to exist - for example, the voltage-gated proton

conductance mediated by H_v1 [62]. Whether there exist any autophagosome-specific conductances is not known.

Cell-type-specific compartments

Whole-endolysosome patch-clamp methods can be employed to study ion channels in lysosome-related-organelles. For example, the albinism-causing OCA2 proteins are reported to encode a Cl- channel in melanosomes that are important for pigmentation [63].

Endoplasmic Reticulum

No K^+ concentration gradient is thought to exist across the ER membrane, and the ER $\Delta\psi$ is around 0 mV [2]. The only major concentration gradient across the ER membrane is for Ca^{2+}, suggesting that a major function of ER ion transport is Ca^{2+} signaling. Free $[Ca^{2+}]_{lumen}$ in the ER is 0.3-0.7 mM, which is established and maintained by the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) [64]. Although there are no concentration gradients, the flux of other ions such as H^+ and K^+ under certain conditions may regulate Ca^{2+} release and uptake (SI Ref. [9]).

Nuclear patch-clamping

Because the outer membrane of ER is continuous with the nuclear membrane, studying ER channels has been made possible by developing a nuclear patch-clamping method (SI Ref. [8]). Several configurations can be achieved, including nucleus-attached, luminal-side-out, cytoplasmic-side-out, and nucleoplasmic-side-out (SI Ref. [8]). Whole-nucleus configuration would be tremendously helpful in studying macroscopic currents, but has not been reported yet.

ER Ca^{2+} channels

There are two major ER Ca^{2+} channels in mammalian cells (see **Fig. 1**). Localized on the ER and nuclear membranes, the ubiquitously-expressed IP_3-Rs (IP_3-R1-3) are large conductance Ca^{2+}-permeant channels. IP_3Rs are activated by the second messenger $InsP_3$, which is generated upon activation of PLC-coupled receptors on the plasma membrane by extracellular agonists (SI Ref. [8]).

RyRs (RyR1-3) are the second class of ER Ca^{2+} channels that are activated upon opening of DHPRs in the sarcolemmal membranes to amplify the Ca^{2+} signals [65]. Alternatively, RyRs can be activated directly by Ca^{2+} in cardiac muscle cells and neurons [65]. While endogenous RyRs are studied mostly by reconstitution into the lipid bilayer, overexpressed RyRs are studied using nuclear patch-clamping [66, 67].

Several non-selective cation channels, including TRPP2, TRPV1, TRPM8, presenilins, mitsugumin23, and pannexin channels are also found in the ER/SR membranes of various cell types and are proposed to be the ER Ca^{2+} leak channels [2]. Whereas confirmation from nuclear patch-clamping is still lacking, Ca^{2+} imaging studies have demonstrated the roles of these proteins in passive depletion of the ER reservoir [68] .

ER K^+ channels

ER $\Delta\psi$ is negligible, thus cation influx is not driven by a luminal negative potential. However, several studies point to functional expression of different K^+ channels and a K^+-H^+ exchanger in the ER membrane (**Fig. 1**). Most of these K^+ channels/transporters are not ER-specific, and are located in the plasma membrane and other intracellular organelles such as mitochondria. These include the ATP-sensitive K^+ channel (in PM and mitochondria), the small and large-conductance Ca^{2+}-activated K^+ channels (present in PM and mitochondria), and the mitochondrial K^+- H^+ exchanger KHE (presumably formed by LETM1 present in the ER and mitochondria) [69] (see **Fig. 1**). The monovalent cation-permeable trimeric intracellular channels (TRIC channels) are expressed in the ER/SR as well as in the nucleus of myocytes [69]. Two proteins located in the nuclei of plants, Castor and Pollux, have been shown to form K^+-permeable channels (see **Fig. 1**) when reconstituted in the planar lipid bilayer [70]. Both proteins are required for the initiation of nuclear Ca^{2+} spiking [70].

The prevailing view is that ER K^+ channels, along with ER Cl^- channels of the CLC family (see **Fig. 1**) might ensure rapid counter-ion fluxes across the ER/SR to compensate for the charge movements associated with Ca^{2+} release and re-uptake processes ([68] ; SI Ref. [9]). During the Ca^{2+} uptake phase, SERCA extrudes protons from the ER, which can re-enter the lumen via the KHE (SI Ref. [9]). In turn, this would lead to an asymmetry in K^+ concentration, which would be re-adjusted following entry of K^+ into the lumen via the

aforementioned K^+ channels. K^+ re-entry also facilitates H^+ entry and K^+ export via KHE, fostering the activity of SERCA2 (SI Ref. [9]). In addition, the observed voltage sensitivity of the ER/SR K^+ channels suggests that these channels might "clamp" $\Delta\psi$ close to zero mV [68] . Finally, ER K^+ channels may also control the volume of the ER lumen, thereby modifying functional properties of this organelle (SI Ref. [9]).

Yeast mechano-sensitive channels

Yeast ER membranes may express mechano-sensitive channels that are activated by hypo-osmolarity (SI Ref. [25]). Mechanosensitive channels are also expressed in yeast vacuoles, in which TRPY1 is activated by membrane stretch and Ca^{2+} [71]. As membrane curvature is expected to generate force during membrane fusion and fission processes of organelles, mechano-sensitive channels may play important roles in membrane trafficking. However, mechano-sensitive channels in the intracellular organelles of mammalian cells have not been reported.

Golgi apparatus

Because the Golgi apparatus receives input from ER-derived vesicles, many of the ER channels and transporters are also localized in the Golgi apparatus, including IP3Rs and SERCA pumps. However, there are also specific Ca^{2+} transporters in the Golgi apparatus, including SPCA pumps [72]. Development of direct patch-clamp methods on isolated Golgi apparatus may promote functional characterization of Golgi-specific channels.

Mitochondria

The existence of ion-conducting pathways in mitochondria has been long-known from classical bioenergetics studies. The channel activities in mitochondria have been observed during the last 30 years either by patch-clamping isolated mitochondria and mitoplasts devoid of their outer membrane, or by incorporating mitochondrial membrane vesicles or purified native/recombinant proteins into planar lipid bilayers [69] .

Due to the highly negative membrane potential in mitochondria (-150 mV to -180 mV), a strong driving force exists for ion movement through ion channels in the inner

mitochondrial membrane (IMM). Since oxidative phosphorylation requires an electrochemical gradient across the IMM, ion channels in this membrane are expected to play an important role in the regulation of energy metabolism. Indeed, the channels operating in the IMM are highly regulated in order to avoid imbalances in energy transduction and consequent processes, e.g. increased production of reactive oxygen species. In fact, as illustrated by some reviews in this SI [17, 18], disturbance of mitochondrial ion homeostasis and/or membrane potential by affecting channel activity leads to severe mitochondrial dysfunction with consequent metabolic changes and/or cell death.

Mitochondrial conductances

The mitochondrial channels characterized over the past three decades include the voltage-dependent anion channel (VDAC) in the outer membrane (see **Fig. 1**). In the inner membrane, the list includes K_{ATP}, Ca^{2+}- activated large, intermediate and small-conductance K^+ channels, Kv1.3, the TWIK-related acid-sensitive K^+ channel-3 (TASK-3), the nonselective permeability transition pore MPTP, chloride channels, the magnesium-permeable Mrs-2, the calcium uniporter MCU, and uncoupling UCP proteins (see **Fig. 1**) (for recent reviews see e.g. [69]). Interestingly, the single channel conductances range from a few pS (MCU), to the nS range (MPTP). Despite the successful introduction of large-scale proteomics into the mitochondrial channel research, molecular identification of these channels is still incomplete. Mitochondrial channels are encoded by the nucleus and in most cases do not harbor clear targeting sequences. In addition, their low abundance and high hydrophobicity render proteomic identification extremely difficult. Nevertheless, the Mitocarta compendium, an inventory of more than 1000 proteins with proven mitochondrial location [73], significantly moved the field ahead, by allowing identification of some channel modulators and/or components. It must be mentioned that in addition to the channels observed by electrophysiology of mitochondrial preparations, some proteins, known to give rise to channel activities in other membranes (e.g. the vacuolating toxin VacA, a nicotinic acetylcholine receptor, a glutamate receptor family member) have been discovered to reside in mitochondrial membranes as well [69]. However, whatever channels they form in the IMM need to determined.

Mitochondrial Ca^{2+} channels

Ca^{2+} uptake across the IMM is performed by the mitochondrial Ca^{2+} uniporter (MCU) and possibly by mitochondrial ryanodine receptors (mitoRyR). On the other hand, Ca^{2+} efflux is mediated by both Na^{+}-dependent (mitoNCX) and Na^{+}-independent Ca^{2+} transporters (see **Fig. 1**). Mitochondrial calcium homeostasis has received particular attention due to its regulatory roles in the aerobic metabolism and cellular signaling under both physiological and pathological conditions [69]. The long-sought calcium uniporter, characterized in bioenergetic studies, was identified as a highly calcium-selective ion channel observed in mitoplasts in a seminal work [74]. Recent discovery of a variety of molecules impacting mitochondrial calcium uptake supports the emerging view that the uniporter is a protein complex rather than a single protein. However, the exact components of this complex, as well as of the factors determining its assembly, are highly debated and represent a hot topic in the field. A 40 kDa protein named MCU, when expressed in recombinant form *in vitro*, is able to form calcium-selective ion channels [14, 15]. However, some characteristic features of the mitochondrial Ca^{2+} uptake machinery (e.g. the observation that mitochondrial Ca^{2+} uptake varies greatly among different cells and tissues and that the channel displays low activity at resting state but an increased activity after cellular stimulation) are due to the important contribution of several modulators of the channel-forming protein. Indeed, the uniporter is likely a complex composed of an inner-membrane channel (MCU and MCUb, a dominant-negative subunit) and regulatory subunits (MICU1, MICU2, MCUR1, and EMRE) (for recent reviews see e.g. [69, 75]). In particular, both MICU1 and MICU2 are regulated by calcium through their EF-hand domains, thus accounting for the sigmoidal response of MCU to $[Ca^{2+}]_{cytosol}$ in situ and allowing tight physiological control. At low $[Ca^{2+}]_{cytosol}$, the dominant effect of MICU2 largely shuts down MCU activity; at higher $[Ca^{2+}]_{cytosol}$, the stimulatory effect of MICU1 allows the prompt response of mitochondria to Ca^{2+} signals generated in the cytoplasm. In a recent study the whole-mitoplast calcium current was found to be different in mitochondria isolated from different types of tissues [76]. The study of the expression of MCU complex members by quantitative proteomics in different mouse tissues reveal significant differences in the various tissues in the MCU/MICU1 as well as MICU1/MICU2 ratio (SI Ref. [16]), pointing to the possibility of tissue-dependent activity/composition of the uniporter complex.

Posttranslational modifications, e.g. by the calmodulin-dependent kinase II [77], might also account for the differences in MCU activity.

Mitochondrial K^+ channels

As mentioned above, IMM K^+ channels recorded by patch-clamp include calcium-dependent K^+ channels (K_{Ca}), Kv1.3, and TASK-3 (see **Fig. 1**). Although not all channels are recorded in all tissues, most of these channels have wide tissue-expression profiles. With the exception of K_{ATP} that is thought to differ from its plasma membrane counterpart, the K^+ channels found in the IMM display biophysical, biochemical, and pharmacological characteristics resembling those of the correspondent plasma membrane channels, leading to the assumption that the protein entities are the same. Therefore, the generation of genetic models (cells or animals) exclusively lacking the IMM channels is a challenging task. In some cases, for instance, mitoKATP, a definitive molecular identification has not been achieved. MitoKATP has received much attention since its activity has been linked to ischemic preconditioning, ischemic postconditioning, and cytoprotection in general. The confounding non-specificity of available pharmacological agents and antibodies have hampered efforts to identify this long-sought channel at a molecular level (see e.g. [78]). Recently, a short form of the renal outer medullary K^+ ROMK channel (ROMK2 or Kir1.1b) has emerged as a possible candidate [79], but this identification is still under debate.

The mechanisms underlying dual/multiple targeting is still unclear for most mitochondrial channels, as is the case for the ER channels (see above). One exception is BK_{Ca}, which is located on the plasma membrane, Golgi, ER, and mitochondria [80]. A recent study found that mitoBK_{Ca} in the heart is encoded by a splice variant of the *KCNMA1* gene that encodes plasma membrane BK_{Ca}. A 50-aa splice insert is essential for its trafficking to the mitochondria [81]. K^+ channel subcellular targeting may also depend on intrinsic characteristics of the protein such as the length and/or amino-acid sequence of transmembrane segments, as elegantly demonstrated for a viral K^+ channel (SI Ref. [24]).

Modulation of IMM K^+ channels causes changes in ROS production and oxidative phosphorylation capacity, suggesting a role in fine-tuning the oxidative and metabolic state of the cell [82]. For example, inhibition of mitoKv1.3 by membrane-permeable blockers

results in increased ROS production and selective induction of apoptosis in cancer cells *in vivo*, whereas membrane-impermeable Kv1.3 inhibitors are without effect (SI Ref. [17]).

Mitochondrial permeability transition pore

Another mitochondrial channel that has a crucial, well-documented influence on mitochondrial function is the permeability transition pore (MPTP; SI Refs. [18] & [69]). Persistent MPTP opening leads to the loss of $\Delta\psi$ and mitochondrial integrity, ultimately causing cell death. MPTP has been shown to correspond to a high-conductance channel recorded by patch-clamp in the IMM. Recently the ATP synthase has been proposed to be a crucial component of MPTP by several groups [83, 84]. According to one study, MPTP may form at the interface between two adjacent FO domains of the ATP synthase in a dimer [84]. However, in another study, the pore-forming part is the c-subunit ring of the FO of the F1FO ATP synthase [83]. Although no consensus has been reached on the exact way of MPTP formation by the ATP synthase complexes, these findings open new perspectives for several pathologies that are influenced by MPTP activation. The signaling pathways leading to the transition from an energy-conserving to an energy-dissipating device are of great importance in the context of cell survival. Indeed, MPTP modulation can be exploited e.g. by cancer cells to increase their chemo-resistance (SI Ref. [18]). Hopefully, better understanding of the pore structure and function will help design MPTP-active compounds to treat cancer and degenerative diseases.

Chloroplasts

Chloroplasts have a double-membrane envelope as well as internal membrane structure called thylakoids, where photosynthesis and ATP production take place (**Fig. 1**). The outer envelope membrane is considered to be permeable to most ions and metabolites. In contrast, the inner envelope membrane and the thylakoids harbor numerous selective ion and metabolite transport pathways, allowing regulation of optimal metabolic activities and of signaling within this organelle [22, 85] and SI Ref. [86]). A multidisciplinary approach exploiting modern genetics, plant physiology, biophysics, biochemistry and proteomics represents one of the new frontiers in chloroplast research. Indeed, recent

results pinpoint ion homeostasis within the chloroplasts as the master regulator of photosynthesis, as illustrated by the paper of Finazzi et al in this SI [22].

Chloroplast conductances

Several different solute transporters and chloride, potassium, and divalent cation-selective ion channels have been identified, either directly in chloroplast membranes using the patch-clamp technique or after reconstitution of purified envelope membranes or thylakoid vesicles into the planar lipid bilayer (for reviews see [85, 87]). Unfortunately, not all techniques are suitable for chloroplasts of the model plant *Arabidopsis*, for which many knock-out mutant lines are available. While patch clamping of pea chloroplast is technically demanding but feasible [88], to our knowledge, this technique has not been successfully applied to *Arabidopsis* chloroplasts.

As a result, many cases of molecular entities giving rise to chloroplast conductances are unknown. However, excellent mass spectrometry studies became available on chloroplast sub-membranes as well [89] (also see SI Ref. [23]), leading to the discovery of many transporter proteins within this organelle. Intriguingly, only few *bona fide* channels were revealed by this technique. Luckily, even though activity is still not experimentally proven for many new candidates emerging from the high-throughput approaches, sequence analysis and homology searches would allow predictions of their functions, as in the case of the plant counterpart of the MCU [1]. Thus, knock-out plants might be used to unravel their importance for the metabolism/ion homeostasis of chloroplasts. In addition, when looking for ion channels with possible chloroplast location, the cyanobacterial origin of these organelles can be informative and evolutionary conserved proteins might become good candidates (for recent reviews see [90]).

In the few cases in which molecular identification of chloroplast channels was successfully achieved, precious information was obtained on the physiological roles of these channels by using knock-out *Arabidopsis* plants. For example, small mechanosensitive channel-like (MscS-like) *Arabidopsis* homolog AtMSL3, was shown to rescue the osmotic-shock sensitivity of a bacterial mutant lacking MS-channel activity [91]. Localized in the envelope, AtMSL3 has been shown to control plastid size and shape, to protect plastids from hypo-osmotic stress, and serve as component of the chloroplast division machinery [91].

Two-pore potassium channel TPK3, located in the thylakoid membrane, was shown to be a crucial player in the optimization of photosynthesis, by influencing the proton motive force (see [92] and SI Ref. [23]).

A plethora of ion transporters are also present in the chloroplast, allowing the transport of metals, inorganic anions, calcium, and potassium (see Table I in [23]). The function of these transporters range from osmoprotection and protection from oxidative stress to ammonium assimilation. Likewise, many metabolite transporters are expressed in the chloroplast, participating in photorespiration and nitrogen/sulfur metabolism (see SI Ref. [86]).

Plant vacuoles

Vacuoles are the plant counterparts to lysosomes. Therefore, like lysosomes, plant vacuoles accommodate high levels of hydrolytic activities, and store high concentrations of Ca^{2+} and Na^{+} [93]. Unlike lysosomes, plant vacuoles are large organelles, since they occupy 80 -90% of the size of an adult plant cell with a diameter of 20 to 40 μm. This large size has made vacuoles interesting for many electrophysiologists since the beginning of the patch-clamp era [29]. The vacuole fulfills many diverse roles, such as the temporary storage of solutes or potentially toxic compounds. A plethora of transporters and channels are characterized on the vacuolar membrane (also called tonoplast). Many of them have been molecularly identified during the last 10 to 15 years. Here we will focus on transporters and channels involved in Ca^{2+}, Na^{+}, K^{+}, and anion transport. A more detailed and exhaustive overview is given in some recent reviews [29, 94] .

Vacuolar Ca^{2+} transporters and channels

The vacuole is the major Ca^{2+} store and it is generally assumed that Ca^{2+} released for signal transduction is mainly from the vacuole. This assumption is made based on the experiments performed by Alesandre et al. [95] showing that InsP3 releases Ca^{2+} predominatly from the vacuole. Later experiments by Lemtiri-Chlieh [96] and Munnik and collaborators [97] indicated that in plants inositolhexa*kis*phosphate (InsP6) plays a much more important role in intracellular signal trandcution than InsP3. However, the channels releasing Ca^{2+} have remained unidentified. TPC1 proteins may act as vaculor Ca^{2+} channels

(see **Fig. 1**), with conflicting evidence in favor and against its Ca^{2+} conductivity [93]. The propagation of the salt-stress induced Ca^{2+} waves are dependent on TPC1 in Arabidopsis [98], suggesting that TPC1 may indeed act as a Ca^{2+} channel under certain *in vivo* conditions.

Ca^{2+} is taken up into the vacuole likely by two P-type Ca^{2+} pumps as well as a small gene family encoding calcium-proton exchangers (CAXs; see **Fig. 1**), which exhibit a high sequence homology to their yeast counterparts residing also on the vacuolar membrane [99]. Interestingly, different CAX proteins exhibit slight differences in their substrate recognition, with a subset of them transporting not only Ca^{2+}, but also heavy metals such as Cd and Mn [29, 100] .

Vacuolar Na^+/K^+ channels and transporters

The vacuole lumen is iso-osmotic to the cytosol, since the vacuolar membrane is permeable for water [29, 93]. Plants store a large amount of inorganic ions as osmolites within the vacuole [29, 93]. This is energetically favorable compared with the production of organic compounds such as glucose or sucrose. Sodium and potassium are taken up into the vacuole by K^+/Na^+ proton antiporters of the NHX family [101] (see **Fig. 1**). The first NHX was identified as a Na^+/H^+ antiporter [102]. During the first years after this discovery, it was thought that NHXs act as Na^+/H^+ antiporters to detoxify sodium [102]. Later studies demonstratd that vacuolar NHXs, as well as NHXs from the secretory system, mediate also potassium uptake into the vacuole [103].

Several Na^+/K^+ channels have been shown to reside in the vacuolar membrane. The aforementioned vacuolar TPC1 is also likely to be involved in sodium export, mainly in response to signaling cues, since this channel is activated by Ca^{2+}. Furthermore, four out of five members of the TPK family (see **Fig. 1**) are localized in the tonoplast in Arabidopsis [104]. The most well characterized TPK is AtTPK1, whose activation is Ca^{2+}-dependent. The channel activity of AtTPK1 is also stimulated by its interaction with 14-3-3 proteins, but suppressed at high $[pH]_{Lumen} > 6.8$ [105].

Vacuolar anion channels and transporters

Two types of transporters and channels have been described for inorganic anions. In addition, a homologue of the renal carboxylate transporters [106] has been shown to act as a malate transporter in Arabidopsis and citrate exporter in Citrus [29]. The class of ABCC transporters that are presumed to be localized in the vacuolar membrane can transport organic anions [107].

ALMTs have been described first as plasma membrane-localized, aluminum-activated malate exporters [108]. Later, it was shown that members of a ALMT subfamily reside in the tonoplast [108]. Vacuolar ALMTs are permeable to malate, but it is likely that their physiological role is to act as malate-activated chloride channels [109]. ALMTs are specific to plants and have so far not been found in other organisms.

In contrast to animal CLCs, plant CLCs reside exclusively in internal membranes [110]. Two members have been characterized in detail [110]. While CLCc acts as a chloride-proton exchanger, the best characterized plant CLC, CLCa, is a nitrate proton antiporter (see **Fig. 1**). CLCa is required to drive nitrate accumulation in the vacuole. It was shown that a very steep nitrate gradient is maintained in plants accumulating nitrate as a nitrogen reserve [111]. Interestingly, the phosphorylation status of CLCa set the direction of nitrate flux. Hence CLCa is also required, at least partially to unload nitrate [111].

In conclusion, a large number of transporters and channels have been identified in the plant vacuolar membrane. What we lack is more insight into the regulation of this network and how all these transporters and channels interact with each other in order to maintain a cytosolic ion homeostasis.

Future directions

Despite the rapid progress made in the research of organellar channels and transporters, many questions related to subcellular targeting, regulation, structure-function relationships, and physiological roles of intracellular channels and transporters remain. Furthermore, identification of possible ion channel/transporter modulators (e.g. kinases and lipids) in some organelles has begun, opening the exciting possibility of studying organelle-specific regulation of intracellular channels/transporters. The recent exciting insights in organellar channels and transporters will undoubtedly provide further motivation for the scientific community in pursuit of this goal.

Importantly, techniques developed to study one organelle could spark ideas and provide methods to study other organelles. For example, the plant studies have led directly to the molecular identification of new ligand-activated channels in the intracellular membranes of animal cells. This molecular "cross-pollination" exemplifies the importance of establishing a discussion forum in the area of intracellular transport systems: both plant and animal communities must meet and challenge each other with new results and thoughts. Likewise, bringing together scientists working on different organelles will result in innovative ideas and research. For example, it will prove fruitful to repurpose the research and techniques developed in studying lysosomes and mitochondria for the studies of other organelles including autophagosomes, synaptic vesicles, and lysosome-related organelles. For example, whole-melanosome recording has been achieved recently to discover new anion channels in the melanosome [63].

Recent studies reveal that different intracellular organelles (i.e.the ER, mitochondria, and lysosomes) cross-talk with each other to form intracellular networks to regulate basic cell biological processes such as Ca^{2+} signaling, ion and lipid exchange, signal transduction, autophagy, and metabolism. In fact, membrane-contact-sites (MCSs) are crucial for ion transport and lipid exchange between organelles, e.g. ER and mitochondria [112]. The roles of channels/transporters in MCSs, which are difficult-to-study at present, are just beginning to be discovered. For example, the voltage-dependent anion channels (VDACs) in mitochondria were recently shown to interact directly with IP3 receptors in the ER, and this interaction is crucial for cellular metabolism and ATP production [112]. Likewise, ER and endosomes may also cross-talk with each other to regulate Ca^{2+} signaling [113]. In the future we will see more studies on inter-organellar communication and regulation.

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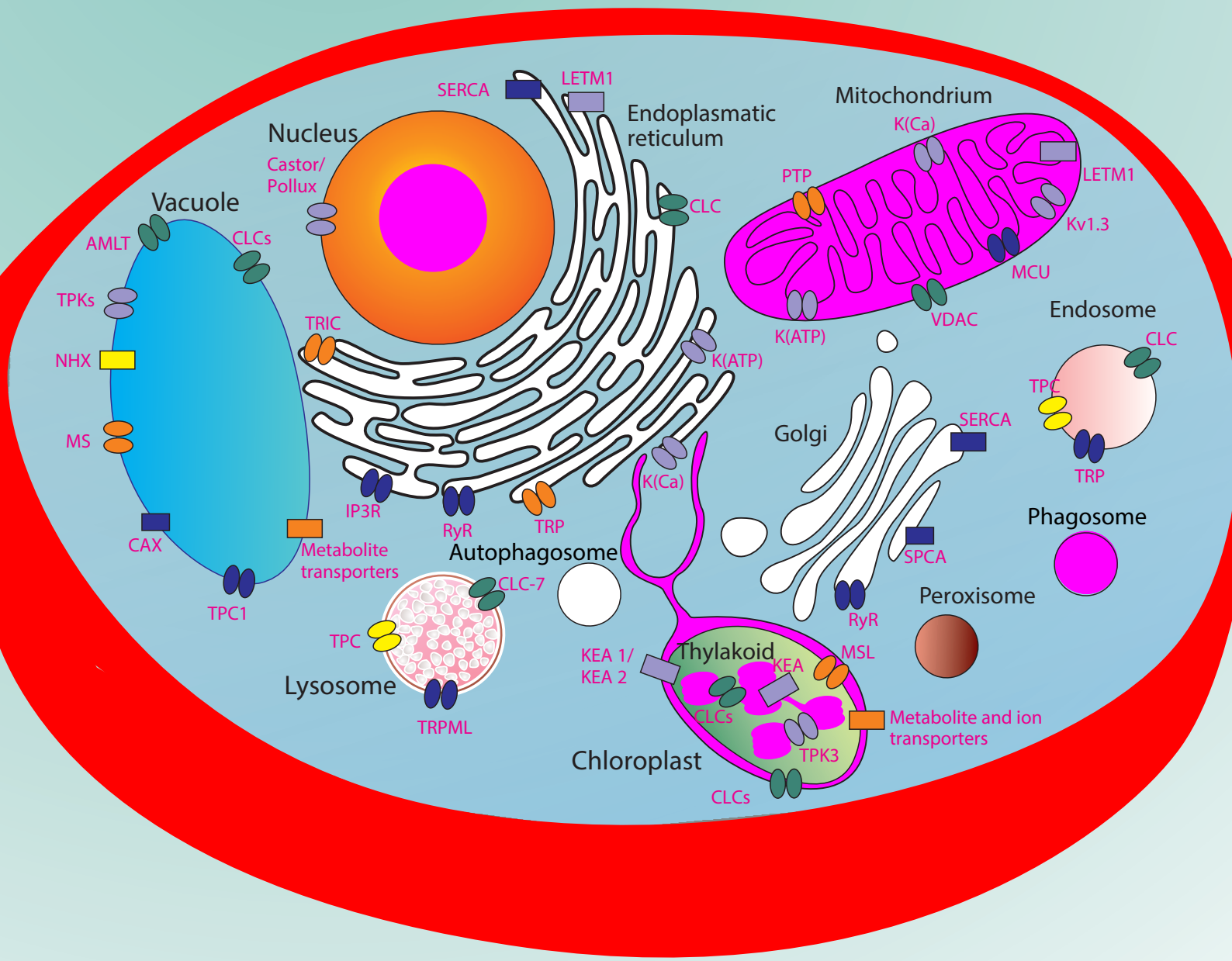
Figure legends

Figure 1. Organellar Channels and Transporters.

Intracellular organelles include endosomes, phagosomes, autophagosomes, lysosomes, mitochondria, chloroplasts, plant vacuoles, Golgi apparatus, the ER, peroxisomes, and the nucleus. Intracellular channels are shown as oval objects while transporters and pumps are rectangular. Channels/transporters are color-coded, with calcium-permeable proteins in blue, chloride in green, sodium in yellow, and potassium in violet. Proteins allowing the passage of metabolites and/or several different types of ions are depicted in orange. In the nucleus of plants, Castor and Pollux proteins may mediate potassium flux. In the ER, several calcium transport systems are found (Ryanodine Receptor, IP3 receptor, SERCA pump) as well as cation-permeable channels (TRIC, TRP). Functionally active K^+ transport systems are the LETM1 K^+/H^+ antiporter, and potassium channels (K_{ATP}, K_{Ca}). In the lysosomes, TRPMLs are permeable to Ca^{2+} and heavy metals; TPCs are Na^+-selective channels, but are also permeable to Ca^{2+}; CLCs are Cl- transporters. TRPs, TPCs, and CLCs are also present in the early endosomes. In the plant vacuoles, TPC1 is the putative Ca^{2+} channels, while CAXs mediate Ca^{2+} uptake. TPKs are vacuolar K^+ channels, while NHXs mediate H^+/Na^+ or H^+/K^+ exchange. CLC proteins function as anion transporters. ALMTs may mediate malate transport. In the mitochondria, only the channels mentioned in this SI are shown - for a complete list see [69]. The MCU complex is responsible for the uptake of calcium. The potassium-permeable pathways in the mitochondria include (K(ATP), K(Ca), Kv1.3 channels, and LETM1 K^+/H^+ antiporter. MPTP is a large, non-specific pore. In chloroplasts, many metabolite transporters

have been identified (see SI Ref. [86]). In addition, ClC-type Cl channel, TPK3 K+ channels, and members of the K^+/H^+ antiporter KEA family have been identified in chloroplasts.


SERCA
LETM1
Endoplasmatic reticulum
Nucleus
Castor/ Pollux
Mitochondrium
K(Ca)
PTP
LETM1
Kv1.3
MCU
VDAC
K(ATP)
CLC
Vacuole
AMLT
CLCs
TPKs
NHX
MS
CAX
TPC1
Metabolite transporters
TRIC
IP3R
RyR
TRP
K(ATP)
K(Ca)
Endosome
CLC
TPC
TRP
Golgi
SERCA
SPCA
RyR
Phagosome
Peroxisome
Autophagosome
CLC-7
TPC
Lysosome
TRPML
KEA 1/ KEA 2
Thylakoid
KEA
MSL
CLCs
TPK3
Metabolite and ion transporters
Chloroplast
CLCs